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Growth and Development of Mice Offspring After Irradiation in Utero with 2,450-MHz Microwaves

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16. ABSTRACT

Mice offspring irradiated in utero with 2,450-MHz radio-frequency (RF) radiation at 0 or 28 mW/cm^2 (whole-body averaged specific absorption rate = 0 or 16.5 W/kg) for 100 minutes daily on days 6 through 17 of gestation were evaluated for maturation and development on days 1, 5, 10, 12, 15, and 17 of age. The tests used to determine differences in developmental age in the two treatment groups were body weight, urine concentrating ability, brain weight, tolerance to ouabain, and bone lengths. Fifteen sham-irradiated and 26 RF-irradiated litters, normalized to eight pups/litter, were used in this study. Mean body weight of the microwave-irradiated offspring were significantly ($p=.0003$) decreased only on day 1 of age. Brain weight on days 10, 12, and 17 were significantly lower in microwave-irradiated pups ($p=.01$). There were no significant differences in the two groups in urine concentrating ability on day 5, ouabain tolerance on day 15, or bone length on days 5, 10, 12, and 17. It is concluded that there is a persistent delay in postnatal development of the brain after RF irradiation with 16.5 W/kg during gestation.

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Growth and Development of Mice Offspring After Irradiation In Utero With 2,450-MHz Microwaves

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ABSTRACT Mice offspring irradiated in utero with 2,450-MHz radio-frequency (RF) radiation at 0 or 28 mW/cm^2 (whole-body averaged specific absorption rate = 0 or 16.5 W/kg) for 100 minutes daily on days 6 through 17 of gestation were evaluated for maturation and development on days 1, 5, 10, 12, 15, and 17 of age. The tests used to determine differences in developmental age in the two treatment groups were body weight, urine concentrating ability, brain weight, tolerance to ouabain, and bone lengths. Fifteen sham-irradiated and 26 RF-irradiated litters, normalized to eight pups/litter, were used in this study. Mean body weight of the microwave-irradiated offspring were significantly ($p = .0003$) decreased only on day 1 of age. Brain weight on days 10, 12, and 17 were significantly lower in microwave-irradiated pups ($p = .01$). There were no significant differences in the two groups in urine concentrating ability on day 5, ouabain tolerance on day 15, or bone length on days 5, 10, 12, and 17. It is concluded that there is a persistent delay in postnatal development of the brain after RF irradiation with 16.5 W/kg during gestation.

There appear to be two levels of responses by the fetus to the insult of exposure to radio-frequency (RF) radiation. The response to higher levels of RF radiation is in the form of overt teratologic symptoms, such as anomalous morphology and death. These effects have been reported a number of times (Dietzel and Kern, '70; Dietzel, '75; Rugh et al., '75; Rugh, '76; Chernovetz et al., '77, '79; O'Connor, '80; Lacy, '81; Lary et al., '82) and seem to appear when the core temperature in the pregnant dam reaches at least 40–41.5°C.

However, a lesser response is seen in the fetus when core temperature in the dam increases to only 40°C or 40.5°C as a result of lower levels of RF-radiation. The form of response to this fetal insult does not appear to contain significant incidences of anomalous morphology or death, and may be limited to decreased fetal body weight (Deichmann et al., '63; Dietzel, '75; McCree and Hamrick, '77; Chernovetz et al., '79; O'Connor, '80; Berman et al., '82a; Jensh, '84) or decreased brain weight (Shore et al., '77).

On its own, a temporary decrease in fetal body weight (i.e., a delay in growth) as a result of RF radiation may not evoke much interest in RF radiation as a teratogenic or fetotoxic agent. However, if growth is delayed for a significant period past birth and through the neonatal stages, then, as a significant index of neonatal condition, it is to be taken seriously (Hurley, '77). Therefore, lowered birth weight that resists normalization with age would represent a more severe demonstration of the fetotoxic potential of RF radiation. This characterization would be of special interest if demonstrable in situations in which there was no or little increase in the dam's core temperature.

We had reported (Berman et al., '82a) that we could consistently cause a decreased fetal body weight in mice using RF radiation exposure regimens that cause temperature excursions in the dam similar to those arising from handling alone. Further, at the end of the experiment, we observed a continuation of lowered body weight at 7 days after birth; the decrease in both fetuses and 7-day-old

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neonates born from irradiated litters was similar, approximately 10% lower than sham-irradiated fetuses and neonates. In light of these and other published reports (Pobzhitkov et al., '61; Bereznitskaya and Rysina, '73; Rugh, '76) of decreased neonatal and adolescent body weight as a consequence of in utero RF irradiation, we examined the possibility that the decreased weight induced by daily in utero exposures to RF radiation might persist past the 7 days observed earlier. The objective of this study was to assess the relative maturity of sham- and RF-irradiated litters, expecting that decreased body weight would be associated with decreased maturity of specific systems. This is the report of the study.

MATERIALS AND METHODS

Groups of 50 young-adult CD-1 mice were time-bred and shipped by the supplier (Charles River Breeding Laboratories, Wilmington, MA) to this animal facility on the second day of gestation (the morning that a copulatory plug was found was considered day 1 of gestation). On the fifth day of gestation, the mice were randomly assigned to groups of 25 sham-irradiated and 25 RF-irradiated subjects, and maintained as treated-untreated pairs. On the sixth day of gestation, all subjects were exposed for 100 minutes to 2,450-MHz RF radiation at power densities of 0 (sham irradiation) or 28 (RF irradiation) mW/cm^2 in clear plastic, ventilated radiolucent boxes, 6 cm on each side (Berman et al., '78; '82a). This exposure was repeated daily through the 17th day of gestation between 0930 and 1530 hours. At other times, the animals were maintained in shoebox-type polycarbonate cages with pine-shaving bedding, with Lab Chow (Ralston Purina, St. Louis, MO) and water ad lib, and with a 12:12 light:dark cycle, ambient temperature of 23–25°C, and relative humidity of 45–55%.

Experimental methods for RF exposure have been described in detail (Blackman et al., '75; Elder and Ali, '75; Berman et al., '78, '82a) but are briefly summarized here. The controlled-environment chamber for RF-irradiated animals was constructed of foamed polystyrene. The chamber was situated in a microwave-anechoic chamber and was irradiated with continuous wave (CW) 2,450-MHz energy at a power density of 28 mW/cm^2. The ambient temperature was 20.2 ± 0.5°C, the relative humidity was 50 ± 5%, and the air flow in the exposure boxes was 0.1 m/sec. The sham-irradiated animals were treated in a foamed-polystyrene environmental chamber adjacent to the anechoic chamber. The temperature-controlled air was circulated through both environmental chambers so that the environmental air was common to both groups. The study was done as three consecutive replicates, each replicate with 25 bred mice in each group, resulting in 75 sham-irradiated and 75 RF-irradiated animals. Each group of 25 bred mice was exposed in a 5-by-5 array of animals. In such an array, the whole-body averaged specific absorption rate (SAR), as determined by twin-well calorimetry (Kinn, '77), is 0.59 ± 0.16 W/kg per 1 mW/cm^2 of incident radiation. At the incident power density used in this study, 28 mW/cm^2, the mean SAR was 16.5 ± 4.5 W/kg (Berman et al., '78). The exposure facility, including the environmental control devices, has been fully described by Ali and Weil ('83).

All bred mice were allowed to deliver naturally. Only litters born on one day of gestation, the 20th day, were used in this study. A single day was chosen to reduce variation in weight due to different lengths of gestation, and for scheduling reasons; the 20th day was chosen because in our laboratory the greatest number (78%) of litters of mice was born on that day. In an attempt to control for variation associated with litter size, only litters with eight or more live pups were used. On the morning of the day of birth, at the time the litter was discovered, litters with more than seven live pups were weighed en masse, the litter randomly normalized to eight, disregarding sex or body weight, and the eight weighed again en masse. Assessments for growth and development were made on equal numbers from each litter through 17 days of age.

Table 1 contains the schedule of tests conducted to determine growth and development in these neonates, the days on which each test was conducted, and the number of pups from each litter involved in each test. The litter was weighed on the first day. On the fifth day of age, the next day on which any measurements were made, the litter was weighed again, and two pups were randomly chosen for the determination of urine concentrating ability. At the end of this test, the same two pups were killed and used for bone measurements. Six pups remained in the litter for use on the tenth day of age. The schedule shown in Table 1 was continued for each

TABLE 1. Schedule of tests at each age and the number of pups used in each litter

Neonatal age (days)	No. in litter before test	Test(s) conducted	No. in litter after test
1	8	(a) Body weight of 8 pups in litter	8
5	8	(a) Body weight of 8 pups in litter (b) Urine concentration of 2 pups, then (c) Bone length of same 2 pups	6
10	6	(a) Body weight of 6 pups in litter (b) Brain weight of 1 pup, then (c) Bone length of same pup	5
12	5	(a) Body weight of 5 pups in litter (b) Brain weight of 1 pup, then (c) Bone length of same pup	4
15	4	(a) Body weight of 4 pups in litter (b) Ouabain lethality of 3 pups in litter	1
17	1	(a) Body weight of 1 pup in litter, then (b) Brain weight of same pup, then (c) Bone length of same pup	0

litter until there were no remaining pups after the tests done on the 17th day of age.

Five tests of growth and development were conducted to aid in the differentiation between sham-irradiated and RF-irradiated groups: body weight, bone length, urine concentrating ability, ouabain tolerance, and brain weight. These tests were chosen on the basis of having certain characteristics: they had measurable changes occurring during the experimental period, the methods had been published in the open literature, techniques were not beyond available skills and equipment, and slopes of age-related changes were relatively steep.

Body weights were determined by weighing littermates en masse. Values were then converted to mean weight per pup, which was the value used for that litter in subsequent statistical analyses. No attempt was made to sex the pups.

Urine concentration was measured in different nontreated CD-1 mice on day 1–10 of age, 3 to 14 mice per day, using a modification of the method of Kavlock (Kavlock, '82; Kavlock and Gray, '82, '83). Pups were removed from their dams; their bladders were emptied by perineal stimulation; and the pups were set aside for 5 hours in beakers, with bedding, floating in a water bath so that the surface of the bedding was maintained at 32–34°C. At the end of this period of deprivation of fluids, the pups were again stimulated manually to urinate. The urine was collected directly into plain microhematocrit capillary tubes, and the tubes were stoppered using capillary tube sealant (Miniseal, Scientific Products, McGaw Park, IL) in both ends of the tubes and then centrifuged. Centrifugation drove one of the stoppers through the length of the tube, ensuring thorough mixing of the sample. Eight-microliter aliquots were measured for osmotic pressure (Vapor Pressure Osmometer, Model 5001B, Wescor, Inc., Logan, UT). Values are reported as mOsm/kg of urine.

For the use of this method in the determination of RF-irradiation effects, urine concentration was measured in 12 sham-irradiated and 23 RF-irradiated litters on the fifth day of age. Pairs of pups were removed from their dams and urine collected as described above except that an equal amount of urine from each of the pair was collected into one capillary tube. The tube was stoppered and centrifuged for even mixing of the two samples and the urine measured for osmolatity. The resulting value was accepted as the value for the litter.

Bone lengths were measured on the 5th, 10th, 12th, and 17th day of age. After the determination of urine concentration on day 5 of age and after decapitation for the measurement of brain weights on days 10, 12, and 17 of age, the carcass of the pup was eviscerated and preserved in 70% ethanol. Later, the carcass was cleared using KOH and the bones stained using alizarin red-S. The lengths of the tibias and radii and the diameter of the blades of the scapulae were determined using a dissecting microscope with a calibrated ocular micrometer. The length of each radius and tibia was the distance between epiphyses. Values for contra-

lateral bones in each pup were averaged; on the fifth day of age, when pairs of pups from litters were used, the averaged value for each bone-type of each pup was again averaged with its littermate, and this value represented the litter value for that measurement.

Brain weight was determined on the 10th, 12th, and 17th day of age. Brain weights were analyzed using analysis of covariance with body weight as the covariate (Edwards and Wanner, '77). Only about half of the litters were sampled for this procedure on each day. This was because the chemical analyses for which they were intended (and which was not completed) was limited to a small number of samples.

Tolerance to ouabain (G-strophanthin) was determined in three pups from litters on the 15th day of age. The technique is a modification of that used by Cagen and Gibson ('77). Each pup in the litter was injected intraperitoneally with 4, 6, or 8 mg ouabain/kg body weight using a 32-guage needle and a microliter syringe. The ouabain was in sterile physiologic saline solution. The pups were returned to their dams and observed 24 hours after injection for survivors and removed from their litters. Each pup contributed individually to the probit analysis and calculation of LD_{50} values, an exception to the manner in which data was handled generally for this study.

The data had a number of characteristics that influenced the choice of statistical analytic method. First, the data were generally the result of measurements of a litter. For example, body weights were determined by weighing the litter and dividing by the number in the litter. In another example, bone lengths of littermates were averaged when more than one pup of a litter was measured on any day (day 5). Also, urine concentrating capacity was measured in two pups in a litter, but averaged by mixing to arrive at a litter value. Second, the same test was often applied repeatedly on the same litter. This was done for body weight, bone length, and brain weight. Third, the data of tolerance to ouabain are different from all the other data in that it is binomial (alive or dead) type data. Further, the expectation of the experiment was that the applied tests of development were related to one another in that they all looked at physiologic or morphologic maturity. However, there is no clear single methodology available which could be applied for statistical analysis of all the data with these characteristics.

Therefore, we used a number of techniques in our examination of the data to try to discern differences between sham- and RF-irradiated mice. All available data were first analyzed as separate variables (body weight, etc.) on separate days. For example, body weights on days 1, 5, 10, 12, 15, and 17 were analyzed separately using the t-test to differentiate the sham- and RF-irradiated groups. The same approach was taken for length of each bone, the sum of the lengths of the three bones, and urine concentration. Where appropriate, correction was made in the acceptable limits of significance using the Bonferroni method, so that, for example, body weight analyses over the six days of measurement required a $P < .008$ for significance instead of the conventional $P < .05$ ($.05/6 = .008$). Probit analyses were used for the data of ouabain tolerance. The second method used linear regression analyses to differentiate sham- and RF-irradiated litters by examining separate variables that were measured repeatedly over the period of the experiment. Two hypotheses were used: that the lines were parallel (H1) and that the means of the lines were similar (H2). Discriminant analysis was the last method, and included body weights, brain weights, bone lengths, and urine-concentrating ability data. Brain weights were also analyzed separately using analysis of covariance.

RESULTS

Three replicates of 50 bred mice were used, yielding 75 sham-irradiated and 75 RF-irradiated bred mice. Thirty-seven bred mice were not pregnant and 113 delivered litters. Twenty-four mice were deleted from the experiment because they had at least one dead pup, mismatched identification, accidental feed or water deprivation, or other experimental accident. The remainder, 89 litters, had similar distributions of lengths of gestation and litter sizes in both treatment groups. However, the RF-irradiated newborn mice were significantly lower in weight (mean ± SEM and number: sham-irradiated = 1.74 ± 0.47 gm, N = 40; RF-irradiated = 1.57 ± 0.57 gm, N = 49; $F(1,88) = 6.48$, $P = .0127$). Of the 89 litters, only 41 (15 sham-irradiated and 26 RF-irradiated) could satisfy the criteria of a 20-day gestation and at least 8 live pups. Table 2 contains the incidences of non-

pregnant mice and the 41 usable litters; the ratios in the two groups were not significantly different.

On the morning of their birth on the 20th day of gestation, litters with eight or more live pups were weighed. Table 3 contains the litter mean of the pup weights before and after normalization. When tested for statistical differences, which included adjustments of weight for litter size, the nonnormalized RF-irradiated litters were significantly smaller than those in the sham-irradiated group ($p = .005$). After normalization to eight/litter, weights were the same as before, though the P-value was slightly lower ($p = .003$). This verified that the pups in the normalized litters were representative of the pups from which they were taken.

TABLE 2. Distribution of usable litters (> 7 live/litter, born on 20th day of gestation, etc.) and nonpregnant bred mice ($X^2 = 1.725$, df = 1, P = .189)

	Power density, mW/cm^2	
	0	28
Pregnant	15	26
Not pregnant	19	18

TABLE 3. Body weights (gm) of newborn mice before and after normalization to 8/litter in 41 usable litters (Values are mean ± SE of litter means)

	Power density, mW/cm^2 (sample size)	
	0 (N = 15)	28 (N = 26)
Before normalization (≥8/litter)	1.62 ± 0.03	1.51 ± 0.02*
After normalization (8/litter)	1.62 ± 0.03	1.51 ± 0.02**

*F (1,38) = 8.81, P = .005.
**F (1,38) = 10.45, P = .003.

We did not feel concern with the possibility that RF-irradiated litters would have contained cannibalized, malformed newborn at a greater incidence than the sham-irradiated litters. Repeated experiments at this exposure level have not demonstrated any increase in terata in rats, hamsters, or mice (Berman et al., '78, '82a, '82b; Berman and Carter, '84). Also, no partially cannibalized neonates were seen in their cages when newborn litters were initially examined.

Body weights were measured on 6 days from 1 through 17 days of age. Table 4 contains these weights. When t-tests were done on the values of each separate day, only the means of day 1 of age were significantly lower in the RF-irradiated pups. Analysis of variance of the data over the 6 days of growth curves (H1) of the two groups yielded no significant difference, nor was there any differences in the levels of the means (H2). Therefore, the pups' growth after the first day of age appears to be the same whether or not they were RF-irradiated in utero. It should be noted that the mean weights were lower at every age in the RF-irradiated group than in the sham-irradiated group.

Bone lengths were measured on days 5, 10, 12, and 17 of age. Figure 1 contains curves for the means of tibial and radial lengths and diameter of the blade of the scapula in each group. The same analytic procedure was conducted on each bone-type as done for body weights, and no statistical differences were found in t-tests of each bone separately on each day, or in the H1 or H2 tests. Additionally, the value for bones in each pup was summed to give one bone value for each pup. However, even this effort to enhance by addition a trend of small differences in one direction did not result in a statistical

TABLE 4. Body weights (gm) of mice offspring exposed to 2,450-MHz microwaves in utero (Values are means of litter means and standard error. t-Value is for analysis on each day)

Age (days)	Power density, mW/cm^2 (sample size)				
	0 (N = 15)		28 (N = 26)		t-value (df = 39)
	Mean	SE	Mean	SE	
1	1.62	0.029	1.51	0.021	3.12*
5	3.55	0.067	3.40	0.054	1.71
10	7.14	0.118	7.03	0.113	0.63
12	8.53	0.158	8.47	0.134	0.28
15	10.24	0.199	10.04	0.197	0.67
17	10.25	0.255	10.23	0.203	0.06

*P = 0.003. H1: F(5,35) = 0.83, p = 0.536; H2: F(1,39) = 0.41, p = 0.533.

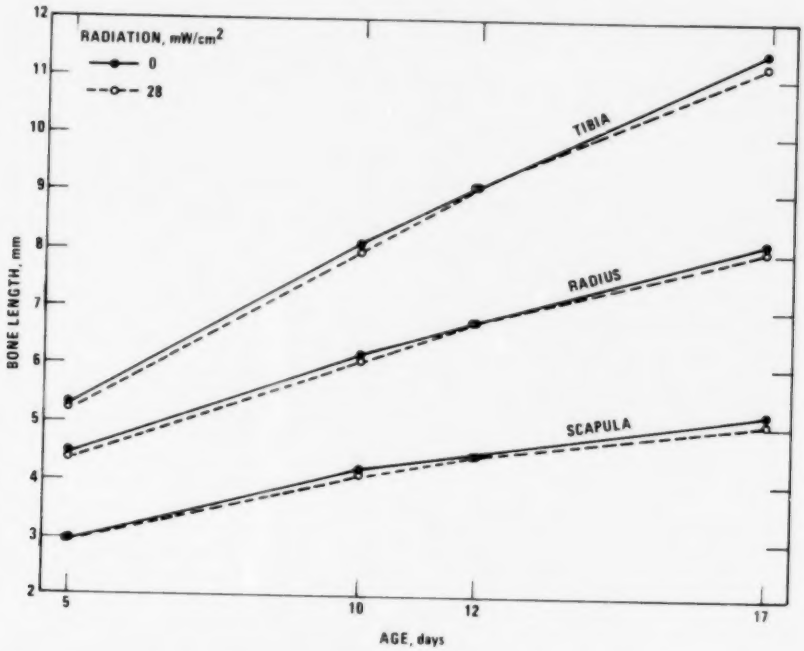

Fig. 1. Means of lengths of the radii, tibias, and blade of the scapulae of neonatal CD-1 mice exposed in utero to 2,450-MHz RF radiation. ●—●, sham-irradiated; ○···○, 28 mW/cm^2.

difference between the sham-irradiated and RF-irradiated groups.

The individual values of brain weights were analyzed using analysis of covariance with its paired value of body weight as the covariate. The adjusted means are in Table 5. The brain weights of the RF-irradiated pups were significantly ($p = .01$) smaller at all three ages (10, 12, and 17 days). The differences of the means at each age were consistent through the experiment (range = 15–16 mg).

*TABLE 5. Mean and SEM of brain weight (gm) of mice offspring after exposure to 2,450-MHz RF-irradiation in utero: Values are adjusted for body weight using analysis of covariance**

Age (days)	Power density, mW/cm^2	
	0 (N = 8)	28 (N = 13)
10	.362	.347
	(.009)	(.008)
12	.398	.382
	(.008)	(.006)
17	.424	.409
	(.010)	(.008)

*F = 7.14 (1,56), P = .01.

Because of the lack of data in the literature relating to the development of the mouse's ability to concentrate urine, we determined a curve of this maturation in CD-1 mice. Figure 2 contains idealized curves representing the means for urine taken before and after a 5-hour period of deprivation of fluids (milk). Until 5 days of age, urine concentration after deprivation appears to parallel concentration before deprivation. At 5 days of age and after, renal maturation enables the neonate to concentrate urine more effectively, 20% more than day 4 and 35% more than day 5. When this measure was applied to the 5-day-old mice in the irradiation study, the values of urine after deprivation in the sham-irradiated and RF-irradiated litters were equivalent (mean, SEM, and N, respectively; sham-irradiated = 697 mOsm/kg, 29.7, 12; RF-irradiated = 698 mOsm/kg, 18.6, 23).

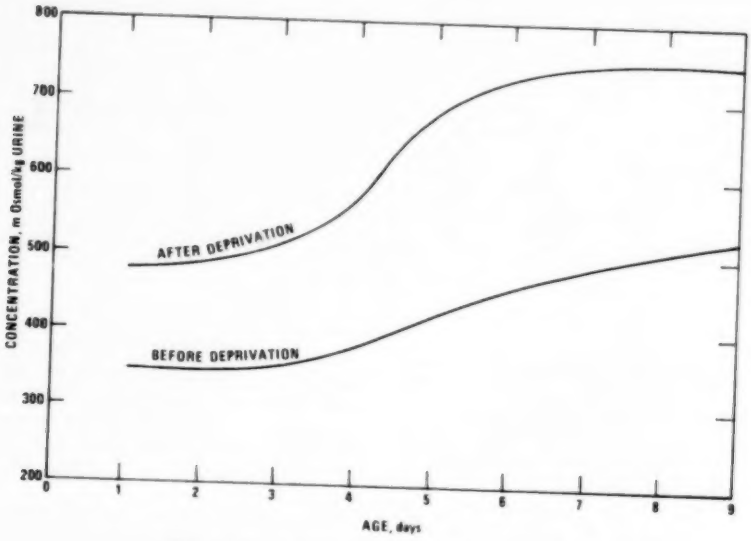

Fig. 2. Urine concentration in neonatal CD-1 mice before and after 5 hours of deprivation of fluids.

TABLE 6. Survival (24 hours) of 15-day-old mice offspring after exposure to 2,450-MHz RF irradiation in utero

Ouabain dose, mg/kg (IP)	Power density, mW/cm^2			
	0		28	
	Dead/live	% Dead	Dead/live	% Dead
4	6/9	40	5/18	22
6	7/5	58	13/9	59
8	11/4	73	20/5	80

Tolerance to an intraperitoneal injection of ouabain in these mice offspring was tested on the 15th day of age. There were a number of failures in the technique of injection in which the ouabain solution was not entirely injected. Only 31 of the 41 litters contributed a pup to each of the three levels of dose of ouabain, and only the data of these 31 litters were used in the analyses. Multivariate analysis of variance techniques did not show any significantly altered death incidences in the RF-irradiated group injected with ouabain (H1 and H2). The rates of survival at the 24th hour after injection is shown in Table 6, and is given in both the real incidence of dead/live pups and in relative terms of the total. The calculated mean LD_{50} was 4.83 and 5.60 mg/kg in the sham- and RF-irradiated groups, respectively.

DISCUSSION

There is a growing concern that conventional tests for teratogenic potential are not adequate for the task of determining fetotoxic influences carried over into postnatal life. It does not appear to be satisfactory to rely only on a demonstration of increased death rate or increased incidence of anomalous morphology as indicator of teratogenic potential in those situations in which environmental pollutants may induce less overt developmental maturation alterations, such as the weight decrement often observed in young mice after exposure to chemicals

TABLE 7. Body weight of mice offspring exposed in utero to 2,450-MHz RF irradiation at power densities of 0 and 28 mW/cm²

Age (days)	Mean weight (gm)		Coefficient of variation (%)		Difference	
	0	28	0	28	Real (gm)	Relative (%)
1	1.62	1.51	6.9	7.1	0.11	7
5	3.55	3.40	7.3	8.1	0.15	4
10	7.14	7.03	6.4	8.2	0.11	2
12	8.53	8.47	7.2	8.1	0.06	1
15	10.24	0.04	7.5	9.9	0.20	2
17	10.25	10.23	9.6	10.1	0.02	0

(Chernoff and Kavlock '82) or to RF irradiation (Rugh, '76; Berman et al., '82a). In light of no clear conventional protocol to discern whether the decreased fetal body weight (Berman et al., '78) and decreased early-neonatal body weight (Berman et al., '82a) induced by RF irradiation in mice is deserving of significant concern, we applied available measurements to exposed animals up to 17 days of age.

We hypothesized that decreased neonatal weight, as an indicator of general neonatal health and normality (Horley, '77), would be accompanied by other symptoms of immaturity. In previous studies of the effects of RF radiation in mice, hamster, and rat fetuses (Berman et al., '78, '82a,b; Berman and Carter, '84), we consistently observed that decreased fetal body weight induced by RF irradiation was also associated with less mature skeletal development. Though significant on the basis of statistical tests, the decrement in both body weight and skeletal maturation were small. Mean body weight in RF-irradiated mice fetuses was not even 10% less than sham-irradiated fetuses. Sternal maturity was also delayed significantly. In our laboratory, cage-control untreated CD-1 mice fetuses have mean and SD values for sternal ossification centers of 4.41 $\pm$ 1.47 at 18 days of gestation and 6.05 $\pm$ 0.12 at 19 days of gestation. Within the framework of the rapid progression of sternal ossification, the small decrement seen as a result of RF irradiation (0.32 ossification centers) represents only a fraction of a day, possibily only four or five hours of gestation.

In order to test the hypothesis, body weight was measured at 1, 5, 10, 12, 15, and 17 days of age. A statistically significant, but small, decrement (7%) in mean body weight was noted on the first day after birth but virtually was gone by the end of the experiment.

It is seen in Table 7 that though the real difference remained to the 15th day, the relative difference became less throughout the experiment. The coefficient of variation also increased steadily and may have prevented the statistical differentiation of the two populations.

An explanation for the different outcomes in this study and that of Berman et al. ('82a) is the treatment of litter size. In the earlier study no attempt was made to normalize litter size, and the means of litter sizes were two to three pups larger than in the present study. By normalizing litter size we meant to remove the influence that litter size has on nutrition and growth during the suckling period. By doing so, we may have placed the eight pups into a more optimum nutritional situation, one in which the smaller, RF-irradiated pups had an opportunity to erase their differences with the sham-irradiated pups earlier than in the 1982 study. Decreasing litter size during the course of the remaining five test days may have also contributed to this better nutritional status.

However, brain weight was significantly different in the RF-irradiated offspring on each of the days it was measured (10, 12, and 17 days of age). The pattern of brain growth in the mouse is directly related to the growth of the body until 14 or 15 days of age, after which brain growth and development is almost nil (Kobayashi, '63; Kobayashi et al., '63). This same pattern is seen in the data of this study, in both the RF- and the sham-irradiated offspring, where the rate of gain of brain weight from 12 to 17 days were less than from 10 to 12 days. However, the rate of brain growth of both groups remained parallel during the 7 days of observation. There was no normalization in the growth of the brains of the RF-irradiated offspring, like that which occurred in body weight. There-

fore, the RF-irradiation induced a persistent (at least for this 17 days) decrement in brain development. Shore et al. ('77) reported decreased brain growth in rats up to 15 days of age after in utero exposure to 2,450-MHz microwave radiation at 10 mW/cm^2 daily for 5 hours. Some rise in maternal temperature might be expected from this exposure regimen, but only of a regulatable degree. Albert et al. ('81) have shown that such levels of exposure in pregnant rats slow brain development (Purkinje cell migration). Brain weight decrements have been reported in animals that are more severely stressed by heat (Edwards et al., '76; Jonson et al., '76). Therefore, slowed development of the brains of RF-irradiated offspring might be expected in the absence of a significant maternal thermal stress associated with the RF-radiation exposure.

But, we cannot conclude that the RF-irradiated offspring were different from the sham-irradiated offspring in their capacity to concentrate urine, in bone growth, and in their tolerance to ouabain. However, when we used a multivariant analysis of variance, we were able to analyze together the data of all ages for body weight, brain weight, bone length, and urine concentration after deprivation. The results indicated that the values for the RF-irradiated offspring were significantly decreased ($p = .022$). However, when the birth weight data were removed from the analysis, leaving only data of 5 days and later, no statistical trends were evident.

In conclusion, we have demonstrated a significant decrement in neonatal weight of mice after gestational irradiation with 2,450-MHz microwaves at an SAR of 16.5 W/kg, and a persistent decrement in brain growth up to 17 days of age. No change was seen in postnatal maturation of the skeleton, liver, or kidneys.

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